Report on two years experience in foot-and-mouth disease vaccine production at the Botswana Vaccine Institute

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Summary: This paper describes the setting up of an emergency foot-and-mouth disease vaccine production Unit in Botswana. No major problems were experienced in producing effective vaccines against SAT 1, SAT 2 and SAT 3 types of FMD virus. The vaccines which were produced were tested in cattle, and in general the bovine Potency values were high. In the field, the vaccines were successful in eliminating outbreaks of the disease in Botswana, and in some of the neighbouring countries. Mention is made of the establishment of a permanent Vaccine Institute capable of producing 21 million doses per year.

INTRODUCTION

In Botswana, a great beef exporting country, one of the major concerns of the Veterinary Services Department is the sanitary condition of the livestock. Organised control of foot-and-mouth disease (FMD) was first started in the early fifties and since 1964, has had recourse to vaccination, amongst other measures. In 1977, the vaccines on the market were found to be inadequate in the control of FMD, so the Government of the Republic of Botswana decided to build and operate their own Institute for the production of FMD vaccine, in collaboration with the French firm: IFFA-Mérieux. In order to face this emergency and to supply Botswana with adequate vaccines in the shortest possible time, they decided to work in two successive phases:

— Phase I: emergency phase, for the study of different strains of FMD virus and the production of limited quantities of vaccine.

— Phase II: building of a permanent Institute, with an initial planned production capacity of 21 million monovalent doses per year.

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Owing to excellent cooperation between the Botswana Authorities and the French technical team and also rapid financing by the EEC, the first experimental vaccine was perfected five months after a Cooperation Agreement had been signed on May 12th, 1978. Six months later, semi-industrial production was under way. Between April 1979 and the early months of 1981, the production capacity was gradually increased from 100,000 monovalent doses to 250,000 doses per week. Phase II equipment will be operational only as of October 1981.

This report aims to describe results obtained so far:

— in the industrial production of FMD vaccine produced from virus strains of specifically African origin (SAT 1, SAT 2 and SAT 3 types);

— in tests made on these vaccines (particularly direct or indirect activity tests), and in-the-field results.

**VACCINES**

Careful serological and immunological studies of numerous virus strains of various types (SAT 1, SAT 2 and SAT 3) originating mainly from Southern Africa have allowed the vaccine strains of FMD viruses which are used in the production of efficient vaccines to be selected. The industrial vaccines thus produced can be bought in the following commercial forms:

— *monovalent vaccine « sensu stricto »*: one vaccine strain of one type (SAT 1, SAT 2 or SAT 3);

— *mixed monovalent vaccine*: association of vaccine strains of different sub-types of the same type;

— *bivalent vaccine*: association of vaccine strains of two different types.

The bivalent vaccine presently used in Botswana is a vaccine which associates strains of types SAT 1 and SAT 2. Each valency represented is itself the association of two vaccine strains. As soon as the Phase II equipment is operational, the preparation of trivalent vaccines associating types SAT 1, SAT 2 and SAT 3 will be possible. The volume of one vaccine dose is 1 ml per valency.

There are five vaccine strains in use at present:

— type SAT 1: Botswana .......... 1/77 (SAT 109)
  Rhodesia ............ 12/78 (SAT 105)

— type SAT 2: Botswana .......... 8/78 (SAT 207)
  Rhodesia ............ 2/79 (SAT 213)

— type SAT 3: South Africa ....... 1/80 (SAT 305)

The SAT 3 strain has been used on an experimental level only, and has proved to be entirely satisfactory.
All FMD viruses are multiplied on cattle tongue epithelia, using Frenkel’s technique, which is specific to IFFA-Mérieux. The multiplication of viruses can be controlled by a complement fixation test: Osler’s method (50% hemolysis) adapted by Roumiantzeff, and through infectivity titration on pig kidney cells. Each vaccine strain has been carefully studied with the purpose of examining different parameters of culture, such as: temperature and duration, oxygenation and stirring. After being clarified and purified, the viruses are inactivated by the combined action of pH, temperature and a chemical agent (such as formaldehyde), and are then concentrated so as to include, in a small volume, a certain quantity of antigen which can vary according to the strains used.

The final phase in vaccine production consists in incorporating, by careful stirring, antigens to the adjuvants classically used in manufacture, intended for cattle:
- aluminium hydroxide (homogenised and adjusted to a pre-established pH);
- saponin (the origin, toxicity and adjuvant power of which have all been previously controlled).

The final pH of the vaccine should be near to 8.

Mixed vaccines are produced from monovalent batches so as to respond, in the most efficient way, to the epidemiological context proper to the countries for which they are intended.

**QUALITY CONTROL OF VACCINES PRODUCED**

All raw materials and chemicals used in vaccine manufacture come from previously tested batches. Vaccine quality control can be split into two categories as follows:
- immediate testing: virus inactivation, sterility, safety and activity of finished product;
- delayed testing: lasting quality of vaccines produced and immunity persistence in inoculated animals.

1. **Inactivation test in cells.**

After action of previously titrated formol on the industrial viruses used to produce one batch of monovalent vaccine, 10 ml of inactivated viral suspension is sampled and put on a culture of pig kidney line cells. The cell layer is examined 48 hours later. After a freezing/thawing cycle, a 48-hour subculture is made. The final examination results in the batch being accepted only if no cytopathic effect is observed on the cell layer.
2. Sterility test.

Bacteriological and mycological sterility of each batch of vaccine is tested on the following media: broth and trypticase soya agar, incubated at +37°C.

The final test is done 14 days later.

3. Specific safety test.

All testing in cattle is done at the Motopi Testing Station, which is located in the North of the country. As soon as Phase II is operational, these tests will be done in Gaborone at the Institute in highly protected testing stables, an integral part of the plant.

All cattle tested are totally susceptible to FMD virus, especially free of seroneutralizing antibodies.

All vaccine batches, monovalent or mixed, undergo this specific safety test:

— Monovalent vaccine: 5 doses of vaccine (5 ml) are inoculated to 3 susceptible animals by the intradermolingual route in about 20 different sites. 10 doses are inoculated by the subcutaneous route into the dewlap.

— Mixed vaccine: 2 susceptible animals are inoculated as above. They are given from 3 to 5 doses of vaccine by the intradermolingual route and from 5 to 10 doses by the subcutaneous route.

The animals are then kept under observation for 6 days and the test is considered as being satisfactory if the following points are noted simultaneously:

— absence of marked and persistent hyperthermia;
— absence of lingual, oral and podal lesions (of FMD origin);
— local reactions in the dewlap should be limited and should not lead to generalised disorders.

As soon as these tests are done in the testing stables of the Phase II plant, they will be in accordance with the European Pharmacopoeia.


In the same way as the safety test, these tests are done at the Motopi Testing Station and call for susceptible cattle:

Direct activity testing.

These tests are done according to the bovine potency technique used by IFFA-Mérieux and recommended by the European Pharmacopoeia, i.e.:
Vaccination by the subcutaneous route of 3 groups of 5 cattle each with a variable dilution of vaccine (1 dose, 1/3 and 1/9 of a dose) with a constant volume of 1 ml. Vaccine dilutions are done with carbonate buffer (pH 8.5) which is immunologically neutral.

Challenge 21 days later by intradermolingual injection of bovine virus homologous to the vaccine strain of 10,000 BID 50% in two different sites of 0.1 ml. Two or three additional susceptible animals are used as challenge controls. The test lasts for 7 days and the animals are kept under clinical observation.

The reading is done by observing podal generalisation lesions.

Since 1980, approximately one batch out of four of monovalent vaccine (from 200,000 to 250,000 doses) has been tested in this way, in order to guarantee the quality of marketed vaccines.

**Indirect activity testing.**

The pig kidney line cell seroneutralization technique is used for this test with « Microtest » plates.

The seroneutralizing titres of the sera of inoculated cattle are expressed by the logarithm of the reciprocal of the serum dilution, before being mixed with the virus, which protects 50% of the wells. This titration of the FMD antibodies is done on the serum of the cattle used in the direct activity test, 21 days after vaccination.

Titrations could also be done on sera of animals which had been vaccinated during vaccination campaigns, both in Botswana and in neighbouring countries. The in-the-field use of the vaccines could thus be evaluated.

5. **Delayed test.**

Experimental protocols are presently being determined in order to be able to evaluate:

— the storage period of vaccines and their efficacy after periods exceeding 1 year;
— the immunity persistence conferred to adult cattle after one primary vaccination followed by one booster vaccination.

**RESULTS**

**A. INACTIVATION**

About 100 batches of vaccine were tested and all of them passed the inactivation test; no residual virulence has been observed as yet.
B. STERILITY

This test, which was done on both monovalent and mixed batches, was done on over 150 batches. All results proved to be satisfactory.

C. SAFETY

Results are summarized in Table I and concern 153 batches of vaccine, all of which proved to be perfectly safe. This represents nearly 15 million monovalent commercial doses. 404 cattle were required for these tests.

<table>
<thead>
<tr>
<th>Vaccines tested</th>
<th>Batches accepted</th>
<th>Batches refused</th>
<th>Total batches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monovalent SAT 1</td>
<td>33</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Monovalent SAT 2</td>
<td>64</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>Monovalent SAT 3</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mixed SAT 1</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Mixed SAT 2</td>
<td>31</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Bivalent SAT 1 &amp; 2</td>
<td>11</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>0</td>
<td>153</td>
</tr>
</tbody>
</table>

D. ACTIVITY

1. Vaccines tested by SAT 1 type homologous challenge.

All results obtained can be found in Table II.

Both SAT 1 vaccine strains used (Botswana 1/77 and Rhodesia 12/78) possess high immunogenic powers. The seroneutralizing antibody response of cattle after primary vaccination with a full dose of vaccine is comparable to that obtained by IFFA-Mérieux.

The 3 control cattle used in each test always displayed widespread generalisation lesions except for one which did not show any podal lesions at all.

2. Vaccines tested by SAT 2 type homologous challenge.

All results obtained can be found in Table III.

Both SAT 2 vaccine strains used (Botswana 8/78 and Rhodesia 2/79 confer high protection to the vaccinated cattle.

Although results with the Botswana 8/78 strain were very good and grouped together for bovine potency, the Rhodesia 2/79 strain gave results which
<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>Vaccine batch No.</th>
<th>Challenge reading results*</th>
<th>Values</th>
<th>Antibodies on the challenge day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 dose 1/3 dose 1/9 dose  Pb</td>
<td></td>
<td>Cattle inoculated with one dose</td>
</tr>
<tr>
<td>SAT 1 Rhodesia 12/78</td>
<td>9004</td>
<td>5/5 5/5 4/5</td>
<td>16.52 2.32</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>0407</td>
<td>5/5 4/5 3/5</td>
<td>11.04 2.67</td>
<td>m = 1.62 N = 5 Sd = 0.277</td>
</tr>
<tr>
<td></td>
<td>0412</td>
<td>5/5 5/5 3/5</td>
<td>10.35 1.74</td>
<td>m = 1.42 N = 5 Sd = 0.205</td>
</tr>
<tr>
<td></td>
<td>1403</td>
<td>5/5 3/5 1/5</td>
<td>4.27 1.85</td>
<td>m = 1.36 N = 5 Sd = 0.313</td>
</tr>
<tr>
<td>SAT 1 Botswana 1/77</td>
<td>91403</td>
<td>5/5 3/5 2/5</td>
<td>5.71 2.22</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>0401</td>
<td>5/5 5/5 3/5</td>
<td>10.35 1.74</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>0405</td>
<td>5/5 5/5 5/5</td>
<td>&gt; 16.52 2.32</td>
<td>m = 1.50 N = 5 Sd = 0.235</td>
</tr>
<tr>
<td></td>
<td>1405</td>
<td>5/5 4/5 2/5</td>
<td>6.93 2.03</td>
<td>m = 1.18 N = 4 Sd = 0.320</td>
</tr>
</tbody>
</table>

* Homologous challenge and results given by the number of protected cattle in relation to the number of those challenged; protection criterion: no podal lesion of foot-and-mouth disease origin.

** Precision factor of bovine potency (Pb).
## Table III

**Vaccines tested by SAT 2 type homologous challenge**

<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>Vaccine batch No.</th>
<th>Challenge reading results*</th>
<th>Values</th>
<th>Antibodies on the challenge day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 dose</td>
<td>1/3 dose</td>
<td>1/9 dose</td>
</tr>
<tr>
<td>SAT 2</td>
<td>9001</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>91510</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Botswana 8/78</td>
<td>91511</td>
<td>5/5</td>
<td>5/5</td>
<td>2/5</td>
</tr>
<tr>
<td></td>
<td>0525</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td>1503</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
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<td>0501</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td>0511</td>
<td>4/5</td>
<td>4/5</td>
<td>2/5</td>
</tr>
<tr>
<td>Rhodesia 2/79</td>
<td>0516</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
<td></td>
<td>0517</td>
<td>5/5</td>
<td>1/5</td>
<td>1/5</td>
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<td></td>
<td>01512</td>
<td>4/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>0528</td>
<td>5/5</td>
<td>3/5</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>1508</td>
<td>5/5</td>
<td>5/5</td>
<td>2/5</td>
</tr>
</tbody>
</table>

* Homologous challenge and results given by the number of protected cattle in relation to the number of those challenged; protection criterion: no podal lesion of foot-and-mouth disease origin.

** Precision factor of bovine potency (Pb).
varied from very good (batches 0501 and 0516) to quite bad (batches 0517 and 0512). The protection rate of cattle vaccinated with a full dose was however always excellent.

All the control cattle used in these tests showed podal generalisation lesions in all feet, except for two animals.

3. SAT 3 vaccine tested by seroneutralizing antibody titration.

Only one batch of SAT 3 type vaccine (OE 401) was produced (vaccine strain SAR 1/80), and its activity only indirectly tested by the titration of seroneutralizing antibodies in susceptible cattle, 21 days post-vaccination.

Out of 5 animals vaccinated, the mean titre was found to be over 1.8. This satisfactory result was confirmed during the in-the-field use of this vaccine, when the Veterinary Authorities of the Republic of South Africa were able to contain an outbreak of SAT 3 type FMD at the beginning of 1980. Eight cattle were tested in the field for their antibody level three weeks post-vaccination: the mean titre was found to be equal to 2.05.

4. Indirect activity testing.

Several tests were carried out on different batches of vaccine on susceptible animals vaccinated during prophylaxis campaigns in Botswana, Zimbabwe (Rhodesia) or the Republic of South Africa. These indirect activity tests were mainly concerned with the SAT 2 type strains, especially the SAT 207 Botswana 8/78 strain.

a) SAT 207 Botswana 8/78 vaccine strain.

In 6 series of tests done in 44 cattle, the average titre of seroneutralizing antibodies after primary vaccination was found to be 2.32.

In 2 series of tests done in 50 cattle, the average after a booster vaccination was found to be 2.32.

b) SAT 213 Rhodesia 2/79 vaccine strain.

In 2 series of tests done in 14 cattle, the average after primary vaccination was found to be 1.90.

c) SAT 109 Botswana 1/77 vaccine strain.

In 3 series of tests done in 28 cattle, the average after primary vaccination was found to be 1.50.

E. DELAYED TESTING

Protocols are being drawn up to establish the lasting quality of vaccines produced.

In the SAT 2 valency, vaccine No. 91510 was tested for activity in January 1980 (Pb ≥ 16.52, see Table III). Five cattle (vaccinated with one
dose) were then challenged 18 months later (in July 1981); all the animals were found to be protected (three controls had generalised lesions). This satisfactory result authorises the vaccine to be stored for a period of at least one year and a half.

The immunity persistence (post-vaccination) revealed that:
— in the SAT 1 valency, in primary-vaccinated and booster-injected animals, there is a slight drop in the antibody level after the 5th month;
— in the SAT 2 valency, a similar test was carried out on a group of 10 animals which had had two vaccine injections at a 2-month interval. Six months after the booster injection, the average antibody titre had very slightly decreased.

Concrete conclusions cannot be drawn from such few results, although it can be thought that immunity persistence, after primary vaccination or booster vaccination, does not exceed six months. This is the reason why bi-annual vaccination (as practised in Botswana) is recommendable.

DISCUSSION

The African SAT type strains could be successfully adapted to Frenkel culture. In comparison to type O, A and C strains, they did not pose any particular problems. The Frenkel/IFFA-Mérieux technique can thus be widely used for virus strains belonging to any of the 7 different types of FMD virus.

A. INACTIVATION

The method using formaldehyde as a chemical agent enabled:
— the immunogenicity of the African viruses used in the production of FMD vaccines at the BVI to be preserved;
— an entirely safe product to be obtained.

B. ACTIVITY

Although there are very few reports on SAT type viruses and in particular on activity tests on vaccine strains, it appeared to us however that some conclusions generally drawn in this literature could be widely criticized:

1. Cattle response to vaccination.

Contrary to what has been reported up to now, the African cattle of Botswana or of various bordering countries (Zimbabwe, Zambia, Namibia, South Africa) respond favourably to vaccination (even primary vaccination). The direct activity test results can prove this: out of 100 cattle that had been vaccinated with one dose and then challenged, only two were not protected.
In-the-field results obtained in the various countries mentioned above furnish yet another example.

In Botswana vaccination twice a year of animals in areas where the disease was prevalent (types SAT 1 and SAT 2) led to the elimination of all centres of the disease. The country has been free of FMD since September 1980 which has allowed meat exports to the countries of the EEC to be authorised again as of June 1st, 1981.

2. Quality of vaccines.

Although generally speaking it has been reported that the SAT type viruses proved to be bad vaccine strains (compared to O, A and C types), it does not seem to us that they behave very differently from other types of virus. For indeed, the bovine potency values obtained are in general very high, with the vaccines tested in such a way complying with the recommendations of the O.I.E., drawn up in March 1975 at the XIVth Conference of the FMD Permanent Commission.

It can simply be stated that SAT 2 type strains are undoubtedly the most difficult ones to work with, as they produce more scattered results. In this respect, two years of work are not enough to provide sufficient data to invalidate or confirm this fact, and another report will have to be made in a few years time.

Finally, as regards the lasting quality of the vaccines, the first results obtained after 18 months for the SAT 2 valency show that it is possible to expect a satisfactory lasting quality which can be compared to that of the vaccines produced with other types of FMD virus. To this end, « bovine potency » type tests will be done on vaccine No. 91510 after 2 years storage.

3. Importance of direct activity testing.

Over a period of 2 years these tests required 360 cattle. Each test costs at least FF 30,000 (as the cattle have to be bought). Despite this high cost price, it would appear to us to be of great importance to increase the frequency of these tests rather than to carry out indirect activity tests to try and evaluate the potency of the vaccines produced.

In fact especially with the SAT 2 type Rhodesia 2/79, we can observe that a high mean seroneutralizing antibody value in cattle vaccinated with one dose does not necessarily imply a high bovine potency: the two examples of vaccines 0517 and 01512 are significant in this respect.

And so, as soon as the Phase II stables are operational, we shall test at least one out of two batches of all monovalent vaccines manufactured. Moreover, the precise knowledge of the bovine potency of a large number of vaccine batches will allow us to produce mixed vaccines whose quality will be better known and more constant throughout the year.
CONCLUSION

After two years experience in the production of FMD vaccine at the Botswana Vaccine Institute, very satisfactory conclusions can be drawn:

— the quality of the vaccines has been proved, both on an experimental scale in the laboratory and on a practical scale in the field, in Botswana and in neighbouring countries;

— the quantities produced have been far beyond the most optimistic expectations: over a period of 2 years, the volume of production has been superior to 15 million monovalent doses (Phase I had been originally designed for a production of 2 million doses per year);

— the vocation of a foot-and-mouth disease regional Institute is asserting itself and despite attempts to increase production, it has never been possible to totally satisfy demand. Over 40% of production has been exported to the following countries: Zimbabwe, Zambia, Namibia, Swaziland, the Republic of South Africa, Senegal and Zaire.

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REFERENCES

(See page 334)